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A STUDY OF FUNGI FOUND IN MILK.

BY H. A. CUMMINS, Professor of Botany; VIOLET C. E. KENNELLY, Department of Botany; and
M. GRIMES, Department of Dairy Bacteriology, University College, Cork.

(PLATES 17 AND 18.)

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A STUDY OF FUNGI FOUND IN MILK.

By H. A. CUMMINS, Professor of Botany; VIOLET C. E. KENNELLY, Department of Botany; and
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(Plates 17 and 18.)

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A survey extending over the first five months of 1929 was made of fungi found in samples of milk examined in the course of a clean milk competition, carried out by the Departments of Dairy Bacteriology and Dairy Farming, University College, Cork.

The milk was plated on Nutrient Lactose Agar, to which sufficient Lactic Acid had been added to give the media a *pH* of 3·5, in order to prevent bacterial growth. The plates were then incubated at 70° F. for a week before examination. It was found that fungi frequently appeared in making cultures from milk, and it seemed desirable that these should be investigated in detail.

This was done in conjunction with the Department of Botany, University College, Cork.

Where inoculation experiments for pure cultures were made on uncooked root-vegetables, the latter were cleansed in distilled water, thoroughly sprayed in a 1/1000 solution of perchloride of mercury, and again rinsed in distilled water. Every precaution was taken to keep the vegetables and utensils free from contamination. The three species most frequently found, occurring in almost all the cultures, were *Oidium lactis*, *Acrostalagmus cinnabarinus*, and *Phoma* spp.

(1) *Oidium* (*Oospora*) *lactis*, Fresen (fig. 1, Pl. 17).—In cultures from milk, it appears as a creamy, white, circular flat growth, with concentric zones. No aerial hyphae, or a few very short moniliform hyphae, are sent up. The mycelium is septate and continuous at first, soon breaking into individual cells. Older growths show a torula-like condition at the edges of the cultures.

Grows on turnip, parsnip, and beet when kept in the dark. The vegetable first gets a dry wrinkled appearance, later a white mycelium appears, and finally rapid deterioration takes place, with pulping of the tissues and evolution of foul-smelling gases. When exposed to the light the erect white mycelium disappears, and the fungus changes to a torula condition, the vegetables becoming a slimy mass. It clots litmus milk thickly in 48 hours, with production of acid and decolourisation of litmus.

(2) *Acrostalagmus cinnabarinus* Corda (*Verticillium cinnabarinum* Reink & Berthold) (fig. 2, Pl. 17).—Frequently appears in cultures as a white, tufty mycelium, later changing to brick-red on all media. The fungus spreads, covering other growths without injuring them. The sterile mycelium closely resembles the mycelium of Phoma before the appearance of fructifications, having a twisted, rather withered appearance. The conidia are held together by mucus, forming a head which, when young, has the white glistening appearance of water droplets, but becomes a brownish-red when mature. Fig. 2A shows appearance under low power. Fig. 2B shows conidial heads under high power. Grows feebly on a wound in beet, also feebly on carrot. When cultured in litmus milk, the appearance of the milk is not perceptibly altered after 48 hours.

(3) *Sporotrichum carnis* (fig. 3, Pl. 17; fig. 1, A and B, Pl. 18).—Found very commonly. First isolated 28/2/29. All specimens found had the characteristic form of two or three small concentric circles, "crusty" or crystalline in appearance, with small droplets, and growing on top of a yeast colony (fig. 1A, Pl. 18), varying from 3·12 mm. to 3·9 mm. in diameter. Cultures were made on the following media in Petri dishes, and kept in a cupboard at a temperature of about 62° F.:—

Medium.	After 7 days.	After 14 days.
Potato Agar	Small felt-like patches scattered over dish.	Sparse growth, scattered, older patches showing a reddish-brown discolouration on under-surface.
Dextrose Agar	Scattered patches, felt-like, no droplets, yellow underneath.	Rounded patches, velvety looking, showing rings of growth. Centres of patches slightly raised, and with reddish-brown discolouration on under-surface.
Bacto-Beef Agar (fig. 1B, Pl. 18).	Small felt-like patches, yellowish and concave on under-surface.	Good felt-like growth, colour turning to a creamy yellow, with bright yellow droplets on older patches. Patches ringed and joined by mycelium not bearing aerial branches. Centres of patches raised, and under-surfaces yellow, shading to orange-brown and concave.
5 % Lemco Agar	Single white felt-like patch, pale yellow on under-surface.	Poor, flat "crusty" (powdery) growth, very flat, with a few scattered droplets, and slightly discoloured on under-surface to a reddish-brown.

Medium.	After 7 days.	After 14 days.
2 % Lemco Agar (fig. 3, Pl. 17).	Very sparse scattered growth, large clear droplets.	Not much advance in growth, very sparse, no discolouration on under-surface.
Krainsky's Dextrose Agar ...	Rather small patches, "crusty," with water droplets, under-surface yellow to orange at centre.	Yellow droplets, and general resemblance to the culture on Bacto-beef.
Czapek's Agar	One small white patch, more "fluffy" than any of the other cultures (*i.e.*, had more aerial hyphae), under-surface yellow.	One solid-looking, wrinkled patch, about the size of a halfpenny, velvety in appearance, no water droplets, creamy colour, with a thin white margin. Underside showed discolouration from pale yellow to reddish-brown.

When cultured in litmus milk, after 14 days there was a thin papery growth, and colour of the litmus was not changed; there was a green colouration on the underside of the fungus. After 27 days the litmus became amber-coloured, a slight purple discolouration remaining on top. This culture was verified by Mr. Brooks, who found it as a common mould on meat in cold storage, and described it in detail in the Trans. Brit. Mycol. Soc., viii, 3, p. 131, and text, fig. 4, 1923. This form is considered as allied to the species Aleurisma Link, as understood by Veullemin (*fide* E. W. Mason).

(4) *Hormodendrum* (fig. 4, Pl. 17).—Of very frequent occurrence. Cultures were made on the same media as Sporotrichum, and examined after 14 days:—

Medium.	Appearance after 14 days.
Potato Agar	Small solid patch, $\frac{3}{4}'' \times \frac{1}{2}''$, felt-like, and olive-green in colour, lightening towards margin.
Dextrose Agar (fig. 4A) ...	Growth similar to that on Potato Agar, but surrounded by a lighter margin of sterile hyphae; conidia 3 or 4 in a chain; much secondary branching of conidia.
Bacto-beef Agar	Growth similar to that on Dextrose Agar.
5 % Lemco Agar	Very strong growth in small patches scattered over the dish, of a darker colour than the first three cultures. Spores not in chains.
2 % Lemco Agar (fig. 4B) ...	Scattered growth; very sparse, the mycelium appearing to grow deeper down into the medium than in the other cultures. Aerial hyphae not so dense as in other dishes, and are more fluffy-looking. Spores in chains of from 4 to 7, and closely resemble Hormodendrum elatum. (Fig. 4B, Pl. 17.)
Krainsky's Dextrose Agar ...	Resembled growth on Potato Agar.

This fungus showed a tendency to grow in water, forming a mycelium resembling Fumago in appearance. This form would not flourish in solid media. When inoculated in a wound in swede turnip and potato, a black discolouration appeared, and examination showed the presence of spores resembling those of Cladosporium, with some mycelium.

When cultured in litmus milk, a thick convoluted mass of the characteristic greenish-black colour was formed, filling half of the tube. Digestion of the milk took place with a little production of acid.

(5) *Trichothecium roseum* Link (fig. 5, Pl. 17; fig. 2, A and B, Pl. 18).—Occurs very frequently, and forms a powdery layer, varying in denseness according to the medium; at first white, later changing to pale pink. The mycelium is creeping, branched, and septate. The hyphae are white, the conidiophores upright, sparingly septate, mostly unbranched, and slightly swollen at the end. The conidia are terminal, formed one after another, but they remain hanging when full grown, forming a head. They are pear-shaped, two-celled, and very slightly constricted at the dividing wall. They are at first hyaline, then pale pink.

When cultivated in litmus milk, spore production is very dense, forming a firm layer on the surface, with the characteristic pink colouration. Digestion of the milk takes place with a slight acid reaction, and with a light-coloured deposit at the bottom of the tube.

(6) *Stysanus microsporus* Sacc. (fig. 6, Pl. 17).—Occurred on two occasions. The heads grow singly in groups, except when cultured on dextrose agar, when loosely panniculate branching was observed, as in fig. 6B, Pl. 18. When young the spores are black, but when old change to a glistening silver grey. Height of growth on Czapek's Agar = 1 m.m.; on 5 per cent. Lemco = ·5 m.m. When cultured in litmus milk, a very strong convoluted growth is observed. There is no production of conidial heads. It gives an acid reaction.

(7) *Mucor racemosus* Fres. (fig. 7, Pl. 17).—Mycelium creeping and dense, the colour varying from dirty yellow to white or grey. Sporangia terminal, on branched or unbranched sporangiophores, variable in size, and brownish-yellow. The sporangiophores are of variable length. The chlamydospores are characteristic, and present both in the mycelium and sporangiophores. They are globular or cask-shaped, and contain glistening spores. The columella is club-shaped. The capsule breaks away when mounted in lactic acid and cotton blue for examination, and leaves a collar round the columella. Clots litmus milk thickly within 48 hours, changing the blue to a pure white, with trace of red colour on the surface.

(8) *Mucor corymbifer* Cohn (*Absidia Lichtheimi* Ledner (fig. 8, Pl. 17).—Isolated on three occasions. It has a whitish-grey loosely matted form spreading over the other growths. The hyphae are long, bending down on coming in contact with the cover of the Petri dish.

The sporangia, though very minute, are visible to the naked eye as small dark specks. The sporangiophores bear a terminal sporangium, and lateral sporangiophores sometimes in the form of an umbel.

Largest sporangia 70μ in diameter — smallest, 10μ–20μ. They are dark brown, and apophyses are present which are at first light coloured, but turning dark with age. Structures in fig. 8A are numerous in the medium. They are

irregular in shape, and a greenish-brown colour, with granular contents. Projections from the main body of the mass grow up into colourless mycelia. We can find no reference to these bodies in the literature available; similar bodies occurred in *M. mucedo* (fig. 10, B and C). When cultured in litmus milk, it coagulated with production of acid, soft curd, and separation of whey.

(9) *Mucor mucedo* Linne (fig. 10, Pl. 17).—A very common form, with dirty white mycelium and dark heads. The branching mycelium in the medium gives off erect, unbranched sporangiophores. Sporangia = 70μ–140μ × 50μ–80μ. Spores, 6μ–12μ × 3μ–6μ. Similar structures to those figured in *Mucor corymbifer* (figs. 8A, 10, B and C) were found, which on transference to fresh medium gave out tubes, as in *M. corymbifer*, growing into the ordinary mycelium.

These chlamydospores (?) appeared again as the culture staled.

(10) *Mucor alternans* Van Tieghem (fig. 9, Pl. 17).—A dirty-yellow growth, low and creeping, not having the usual macroscopic appearance of a Mucor. Sporangia of various sizes were on short stalks, branching as in diagram. Spores = 2μ–3μ × 5μ–7μ. When grown in litmus milk, the curd is coagulated, and the litmus decolourised. There is a strong growth of the fungus on the surface with aerial hyphae 1″–1¼″ high.

(11) *Thamnidium elegans* Link (fig. 11, Pl. 17). — Considered entirely saprophytic. When first cultured it spread rapidly, and infected a dish, opened for examination, with a very strong growth. Young forms are difficult to distinguish from a Mucor until secondary sporangia appear. Spores = 8μ–10μ by 6μ–8μ; being the same size in the primary and secondary sporangia. Secondary sporangia generally fall off intact, and when grown in 2 per cent. sugar solution swelled to 7 or 8 times their original size without showing signs of bursting. When grown in litmus milk, coagulation of the curd with decolourisation of the litmus takes place. The curd is soft, with marked separation of whey, and film on surface.

(12) *Pilobolus crystallinus* was found on two occasions.

(13) *Botrytis cinerea* Pers. (fig. 12, Pl. 17; fig. 3, Pl. 18).—Occurred on three occasions as light greyish-brown tufts. When cultured it produced sclerotia and microconidia on the mycelium, as in fig. 12, Pl. 17. The hyphae are dark green, and the conidia, at first colourless, are later tinged with brown. Sterile hyphae were produced on the ends of the mycelia. It also grew well on potato sections, producing a similar result. Spores, 9μ–12μ × 6μ–10μ, from both kinds of conidia. When cultured on litmus milk, there was a growth on surface, and a bad odour was produced.

(14) *Aspergillus Spp.*—About three specimens of Aspergillus were fairly common; of these, one has been identified as belonging to the *glaucus* group (Thom). Ascospores are necessary for the further identification, and these have not yet appeared.

(15) *A. luteo niger* (Lutz) (fig. 13, A and B, Pl. 17; fig. 4, Pl. 18).—Appeared on one occasion; the mycelium at first white, then golden yellow, and

the heads pass through shades from pale yellow to dark brown. The spores when examined dry are seen to have colour bars and tubercules. These disappear when mounted in fluid, the conidia appearing smooth and globose. Primary and secondary sterigmata are present on spherical vesicular heads. Measurements are given on the diagram. The spores are globular, 5μ in diameter, hyaline when young, later fuscous. When grown in litmus milk, rapid digestion takes place, with strong production of acid. The growth of the fungus is very strong in a thick convoluted mass. This particular species of Aspergillus was recognised to be a rare species when found, and a culture was forwarded to Dr. C. Thom (Washington), who identified it as *A. luteo-niger*. The inference drawn from the appearance of cultures is to the effect that the spores are elastically projected, although the act has not yet been actually observed under the microscope.

(16) *Aspergillus terreus* Thom.—Of frequent occurrence, varying in colour from white to brown, passing through shades of pinkish cinnamon, according to age. Vesicle, 12–18μ, bearing sterigmata in two series, closely packed. Primary sterigmata = 2–2·5μ × 7–9μ. Secondary = 2–2·5 × 5–7μ. Conidia are slightly elliptical to globose, 2·2–2·5μ or 3μ.

This form is common in America, but has not been previously received by Thom from Europe.

(17) *Penicillium Spp.*—Several different species of Penicillium were found, but owing to the largeness of the group no steps have been taken to establish the identity of individual species.

(18) *Phoma Spp.* (fig. 5, Pl. 18).—Two species of Phoma were very common. One species was black, and the other pinkish-brown. A microscopic examination shows light-coloured hyphae with dark spots. These latter proved to be pycnidia, with one or two ostioles, by which the spores were extruded in coloured solution. Further investigations are being carried out on these species.

(19) *Torula Spp.* Pers (black) (fig. 16, Pl. 17).—Was found on one occasion.

(20) *Macrosporium sarcinula* Berk (fig. 14A, B, C, Pl. 17).—Found on one occasion. The hyphae are at first light-coloured, turning a brownish-black, septate, and spreading. The conidiophores are very short and swollen (fig. 15A). The conidia are constricted off in chains, a rare occurrence in this form, hyaline and smooth when young, dark and warted when older. They have a network appearance owing to formation of septa, and are constricted at the dividing walls. Spores very variable in size.

(21) *Stemphyllium botryosum* Wallr. (fig. 15A, B, C, Pl. 17).—Found on two occasions. Very similar to *Macrosporium sarcinula*, but the conidia are single, the conidiophores longer and thinner—may be branched or unbranched. The conidia are not so constricted as in *Macrosporium sarcinula*, and more warted.

Summary.

A survey extending over the first five months of 1929 was made of fungi found in samples of milk examined in the Department of Dairy Bacteriology, University College, Cork, in connection with a clean-milk competition. The following were found:—

(1) *Oidium* (*Oospora*) *lactis* Fresen
(2) *Acrostalagmus cinnabarinus* Corda
(3) *Sporotrichum carnis* Brooks and Hansford
(4) *Hormodendrum* Spp. Bonord
(5) *Trichothecium roseum* Link
(6) *Stysanus microsporus* Sacc.
(7) *Mucor racemosus* Fres.
(8) *Mucor corymbifer* Cohn
(9) *Mucor mucedo* Linne
(10) *Mucor alternans* Van Tieghem
(11) *Thamnidium elegans* Link
(12) *Pilobolus crystallinus* Tode
(13) *Botrytis cinerea* Pers.
(14) *Aspergillus* Spp.
A. glaucus group (Thom)
(15) *A. luteo-niger* (Lutz)
(16) *A. terreus* (Thom)
(17) *Penicillium* Spp.
(18) *Phoma* Spp.
(19) *Torula* Pers.
(20) *Macrosporium sarcinula* Berk
(21) *Stemphyllium botryosum* Wallr.

Many of these fungi had not been previously reported as having been found in milk. Their presence is probably accounted for by contamination of the milk due to the conditions under which the cows were housed, fed, and milked, and to improper cleansing of the dairy utensils.

Type cultures of these fungi have been deposited in the National Collection of Type Cultures, Lister Institute, Chelsea, London.

We desire to acknowledge with thanks the valuable assistance in identification and verification rendered by Dr. E. J. Butler, C.I.E., D.SC., F.R.S., M.B.; Mr. E. W. Mason, M.A., M.SC., and Mr. S. F. Ashby, B.SC., of the Imperial Bureau of Mycology, Kew Green, Surrey; Mr. F. T. Brooks, M.A., Cambridge; Dr. C. Thom, Microbiological Laboratory, Bureau of Chemistry, Department of Agriculture, U.S.A.; and to thank Professor C. Boyle, M.A., PH.D., Dean of the Faculty of Dairy Science, University College, Cork, for facilities afforded for carrying out the investigations.

Plate 17.

FIG.

1. *Oidium lactis* Fresen.
   A = ordinary mycelium with aerial hyphae.
   B = torula condition.
2. *Acrostalagmus cinnabarinus* Corda.
   A = low power, general view.
   B = conidial heads, high power.
3. *Sporotrichum carnis.*
   A = Sporangiophores under high power.
4. *Hormodendrum.*
   A = Form on dextrose agar.
   B = Form on 2 per cent. lemco agar.
5. *Trichothecium roseum* Link.
   A = single conidium budded off, a second being formed at X.
   B = Head of conidia.
6. *Stysanus microsporus.*
   A = solitary heads.
   B = branching evident on dextrose agar.
   C = single head enlarged.
   D = section through coreme, showing fibrous hyphae from which conidial chains spring.
7. *Mucor racemosus* Fres.
   A = Chlamydospores in mycelial hypha.
   B = aerial hyphae with sporangia and chlamydospores.
8. *Mucor corymbifer* Cohn.
   A = structure resembling chlamydospore in medium.
9. *Mucor alternans.*
10. *Mucor mucedo* Linne.
    10 B and 10 C = structures resembling 8A.
11. *Thamnidium elegans* Link.
    A = general structure, low power.
    B = secondary sporangia highly magnified.
12. *Botrytis cinerea* Pers.
    A = general appearance, low power.
    B = same magnified.
13. *Aspergillus luteo-niger* Lutz.
    A = section (transverse) through head, showing vesicle, sterigmata, and some chains of conidia.
    B = spores under high power.

FIG.

14. *Macrosporium sarcinula.*
    Showing condia in short chains, hyaline and smooth when young—later dark and warted.
    A = sporangiophore.

15. *Stemphyllium botryosum.*
    A = general appearance, high power.
    B, C, and D.—Conidia greatly magnified.

16. *Torula* Pers.
    General appearance, high power.

PLATE 18.

1. *Sporotrichum carnis.*
    A = microphotograph of colony 3·12 mm. in diameter.
    B = culture on bracto beef agar.

2. *Trichothecium carnis* Link.
    A = growth of colony on potato agar.
    B = growth of colony on bracto beef agar.

3. *Botrytis cinerea Pers.*
    Culture on potato agar, showing black sclerotia, and dark hyphae bearing conidia.

4. *Aspergillus luteo-niger* Lutz.
    Culture on dextrose agar.

5. *Phoma.*
    Culture on dextrose agar.

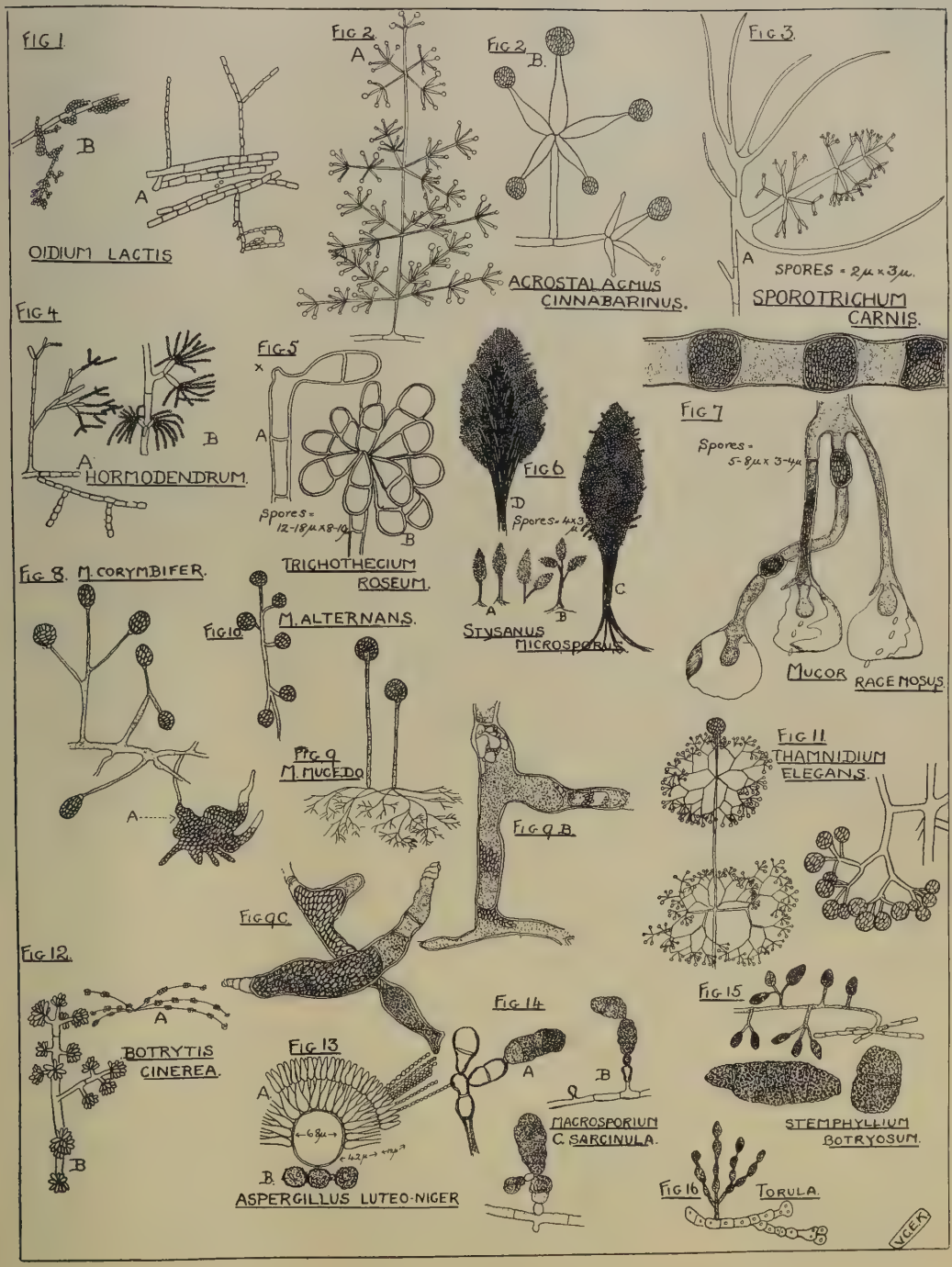
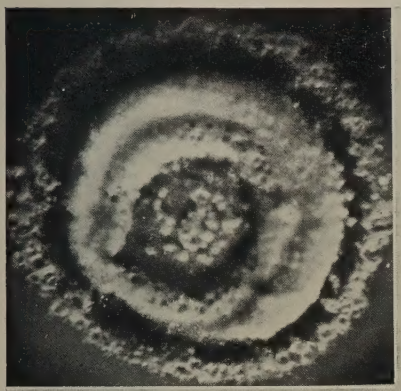
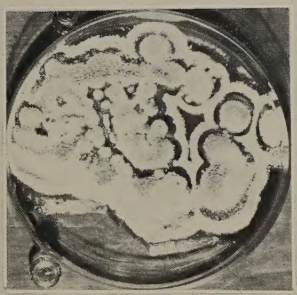
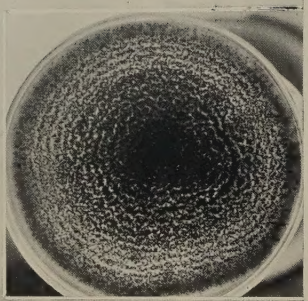


FIG 1
B
A
OIDIUM LACTIS
FIG 2.
A
FIG 2.
B.
ACROSTALAGMUS CINNABARINUS.
FIG 3.
A
SPORES = 2μ × 3μ.
SPOROTRICHUM CARNIS.
FIG 4
A
B
HORMODENDRUM.
FIG 5
x
A
Spores = 12-18μ x 8-10
B
TRICHOTHECIUM ROSEUM.
FIG 6
D
Spores - 4x3 μ
A
B
C
STYSANUS MICROSPORUS.
FIG 7
Spores = 5-8μ x 3-4μ
MUCOR RACEMOSUS.
FIG 8. M. CORYMBIFER.
A
FIG 10
M. ALTERNANS.
FIG 9
M. MUCEDO
FIG 9 B
FIG 9 C
FIG 11
THAMNIDIUM ELEGANS.
FIG 12.
A
BOTRYTIS CINEREA.
B
FIG 13
A
B.
ASPERGILLUS LUTEO-NIGER
FIG 14
A
B
MACROSPORIUM C. SARCINULA.
FIG 15
STEMPHYLLIUM BOTRYOSUM.
FIG 16
TORULA.
VCEK

1. B





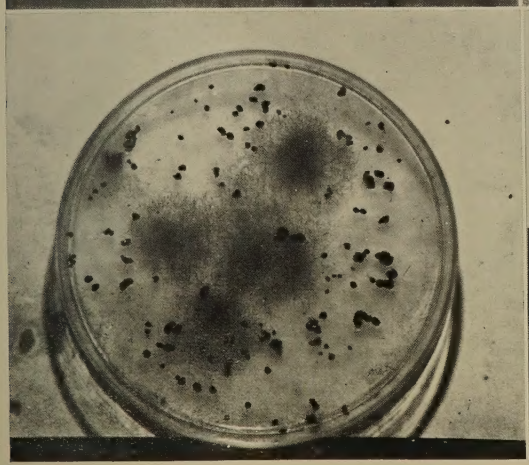


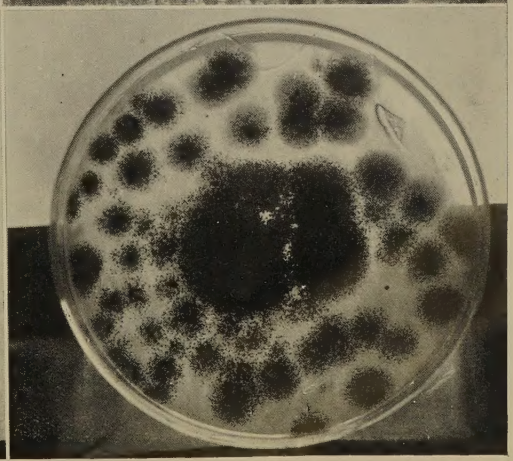
. A

5

2. A

2.





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